REMARKS

Claims to non-elected inventions have been canceled. Claim 13 has been amended as kindly suggested by the Office to replace "and/or" with an alternative phase. The average diameter has been modified to read 100-2000 nm, which is clearly supported on page 20 where 2000 nm is an upper limit and 100 nm is the lowest limit mentioned. Claim 25 has been amended to clarify that the infectious organism is Hepatitis virus. The word "derived from" was not intended to imply modifications.

Claim 15 has been canceled to simplify prosecution.

Claim 16 has been amended simply to specify the origin of antigenic protein and assistor protein.

Support for new claim 27 is found, for example, in Example 6 beginning on page 53.

Support for new claim 28 is found in Example 3.

Claim 29 claims subject matter that the Office has kindly acknowledged as enabled by the specification, and claim 30 is supported on page 20.

No new matter has been added and entry of the amendment is respectfully requested.

Rejection Under 35 U.S.C. § 112, Second Paragraph

Both aspects of this rejection have been addressed by amendment and applicants believe this rejection is obviated.

Rejection Under 35 U.S.C. § 112, First Paragraph, Written Description

This rejection has also been addressed by amendment and clear support has been shown in the specification for the size range indicated.

Rejection Under 35 U.S.C. § 112, First Paragraph, Lack of Enablement

Only claims 15 and 16 were rejected under this statutory section. Claim 13 appears to be free of this rejection as it merely mandates an immune response that would include the antibody response that is demonstrated in the application.

To simplify prosecution, claim 15 has been canceled and claim 16 has been amended to require only the response of claim 13. Much of the art cited by the Office as demonstrating lack of enablement of claim 16 is associated with the difficulty of conferring immunity. There are no documents cited which would indicate that changing the route of administration, the nature of the antigen, etc., using common sense alternatives known to those in the art, would result in a failure of the method of the invention to elicit at least an antibody response. Accordingly, in view of these amendments, this basis for rejection may be withdrawn.

In addition, new claims 29-30 are directed to subject matter acknowledged by the Office to be enabled.

The Rejection Over the Art

All claims were rejected as assertedly obvious over Craig, *et al.*, (WO 97/28818) in view of Gregoriadis, *et al.* (Methods, Vol. 19, pp. 156-162).

While it is correct that Craig proposes the alternative of delivering the same peptide, both in the form of a nucleic acid encoding it and in the form of the peptide itself, it is clear that Craig does not appreciate the necessity of co-delivery or even the necessity of co-delivery of a shared epitope. Applicants recognize that Craig proposes complexes, but also proposes mixtures, and thus, essentially teaches that co-delivery is optional and not necessitated. See page 16 lines 21-25

combined with lines 27-31. Applicants also recognize that Craig permits the peptide antigen and the nucleic acid encoded antigen to be the same; however, again, this is not a necessity, and is not even preferred. Craig states that a more effective immune response may be obtained using a first peptide antigen in combination with a second different nucleic acid encoded antigen. See page 17, lines 4-9. The inclusion of liposomes among many possible delivery vehicles does not mandate or suggest that both the encoding nucleic acid and the protein be associated with the same liposomal delivery vehicle or comprise the same epitope.

According to Craig, the focus of the disclosed "invention" is to ensure that the nucleic acid encoded antigen is expressed only in professional antigen presenting cells (APC) (page 7, lines 4-6) and stipulates that the vehicles must either be directed specifically to APC's or that the expression system be such that expression occurs only in APC's (see page 7, lines 25-30). In the present application target moieties are excluded from claims 13 and 28 by the "consisting essentially of" language, and there is no requirement for APC-specific expression.

In addition, although Craig defines an immune response as including both humoral and cellular responses, it is clear from the prophetic examples, that it is a T cell response that is expected. For example, on page 61, beginning at line 33 and continuing to page 66, all of the measures of eliciting an immune response (in this case, not even *in vivo*) assess T cell responses.

The centrality of ensuring that any combination of the antigenic protein containing a first epitope and an expression system for a second epitope which may be entirely different from the first epitope must be delivered to a professional antigen-presenting cell is verified in the broadest claim, claim 1 on page 67 of Craig. It is also verified by the fact that the only *in vivo* working example set forth in Craig is on page 44 which describes an experiment to verify that an expression system for a

test protein (not even an antigen) can be expressed specifically in professional antigen presenting cells by placing its expression under the control of the mouse MHC Class II Ea gene Locus Control Region (LCR) to assure such expression. In this sole *in vivo* working example the expressed protein and the protein *per se* are entirely different. Even the prophetic examples that describe expression of antigens for infectious agents that follow on page 44-47 are designed merely to confirm that antigen presenting cell-specific expression can be achieved in this manner; the expressed protein and the protein *per se* do not share an epitope.

Thus, the compositions of Craig, which are described only with many alternatives as to delivery vehicles, are specifically designed to target professional APC, not to deliver a common epitope, and not to deliver, necessarily, both the expression system and a peptide simultaneously to the same cell.

The Speculations of Craig Could Not Predict the Invention's Surprising Results

Craig is characterized by a complete lack of any *in vivo* data and merely speculative extrapolation from limited and irrelevant *in vitro* work. Even if Craig's putative examples were to be practiced, this would still not lead to an expectation of success of the claimed co-delivered nucleic acid/assistor protein combination much less a liposomal coentrapped system.

On the other hand the present disclosure demonstrates, using working examples, that liposomally delivered combinations of nucleic acids expressing an antigen and the assistor protein containing a common epitope enhance the humoral response as required by claim 13. This is demonstrated dramatically in the results shown in Example 1; as noted on page 31, at line 27, formulation 1.1 that consisted of both HA DNA and HA protein co-delivered in the same liposomal

formulation produces a greater response than all of the other formulations at each sera sample bleed tested. As noted on page 31, at lines 36, *et seq.*, where the protein co-administered is not cognate to that encoded, the response is not as substantial. As noted in the subsequent paragraph, if separate liposomes are used to deliver the DNA and its cognate protein, the results are not as good.

Contrast this with the disclosure of Craig. There are a few *in vitro* working examples that do not pertain to the present invention as claimed. Some *in vitro* results are described from page 54, line 15 to page 55, line 32. In this experiment, a complex of a peptide, NBC9, and a plasmid encoding green fluorescent protein is used to transfect dendritic cells *in vitro.* There is nothing that happens *in vivo.* The DNA in this example encodes a fluorescent protein unrelated to the peptide administered, which is not an antigenic protein from an infectious organism either. NBC9 is described in W09641606 (PCT/GR96/01396, cited on page 54, line 27). NBC9 is merely a nucleic acid condensing peptide.

The *in vitro* experiments using COS cells described from page 55, line 34 to page 57, line 10, use a different DNA, pCMVb. The nature of this plasmid is not explained. However one might assume, from the tests which are subsequently carried out, that it encodes β-galactosidase. The peptide which is used to complex the plasmid contains an epitope of influenza A nucleoprotein. The nucleic acid does not encode a protein having an epitope of any infectious organism, or even protein which shares an epitope with the complexed protein.

The experiment described from page 58, line 6 is very similar to that described on pages 55 to 57, in that it involves COS cells, the nucleoprotein derived peptide and a reporter gene, in this case encoding luciferase. Exactly what is shown in the results, Figure 5, is not clear. There is a description that what is being determined is a peptide rather than expression

product of the DNA, because the comparison is between 1) complex, 2) peptide alone and 3) buffer. There is no nucleic acid delivered alone, so there is no control to compare the effect of the peptide on transfection by the nucleic acid alone. Instead this experiment seems to show that the peptide penetrates the cell more in the presence of nucleic acid than without, which is not relevant to the present invention, and in any event lacks a crucial control to establish the superiority of the invention method.

The foregoing is the entirety of any working examples presented where both nucleic acid and protein are applied. Since the data from all these examples are from *in vitro* tests, they cannot show any immune response, since this requires an in *vivo* experiment. There is no showing, therefore, that any complex could be delivered *in vivo* nor that, if it were to be delivered *in vivo* it would reach its target, nor that it would, *in vivo,* transfect cells, nor that it would, *in vivo,* transfect antigen presenting cells, still less that nucleic acid would be expressed, still less that there would be an immune response to the expressed protein, still less that this immune response would be higher than the immune response by administering the separate protein and nucleic acid, by the same delivery mechanism, still less that the properties would be improved by co-entrapping the protein and the nucleic acid into the same delivery system, still less into a liposome.

Even the remaining *in vitro* examples are prophetic.

For example, the experiment from page 59, line 20 to page 60, line 32 describes transfection *in vitro* of dendritic cells. The complex which is administered in this experiment is not fully described, only the nature of the peptide. It is clear that this experiment has not in fact been carried out, since from line 10, the methods are merely stated as possibilities. There are no

data presented. Although it is predicted that stimulation of T-cells in this in *vitro* test might be expected, there is no proof of this. How these methods would have any relevance to in *vivo* tests is not explained. It is not even explained how the dendritic cells which might have been transfected by the complex would stimulate the T-cells in the *in vitro* test. Why would dendritic cells that express fluorescent green protein, 13 -galactosidase or luciferase, be capable of stimulating T-cells in this *in vitro* test?

The experiment from page 60, line 34 to page 62, line 35 is also hypothetical. The paragraph from page 60, line 37 to page 61, line 20 concerns only the nature of the complex between the peptide having the DNA binding sequence, and the DNA itself. The tests described from page 61, line 33 to page 62, line 35 are all *in vitro*, and never carried out.

From page 62, line 37 to page 64, line 1, no examples are even described. There are some statements regarding what might be expected to be shown, for instance at page 63, line 25 and line 35, but no data to support these assertions. Although there is reference to an immune response at line 30/31, how this would be assessed is not explained. Nor indeed is it clear why there is an expectation for the generation of an immune response from the data which might be expected from the tritiated thymidine incorporation experiment presumably *in vitro* which is described from lines 24-28. The experiment from page 64, line 3-23 is clearly *in vitro* and speculative. In any event it relates to an additional feature which has no relevance to the present invention.

Thus, in summary, virtually all of the "examples" which involve co-administration of a nucleic acid that encodes a protein (usually not even an antigen) and a protein (often not an antigen either, and never containing the same epitope as that encoded by the nucleic acid) are *in vitro*, and most of them are prophetic.

The Sole Prophetic *In Vivo* Example in Craig Would Not Establish the Superiority of the Invention Method

Finally, on page 64 beginning at line 30, a single prophetic example performed *in vivo* that describes co-administration of a nucleic acid and a protein is set forth. It is suggested that the protein be an influenza nucleoprotein, but there is no description at all of the nature of the co-administered nucleic acid. There appears to be no requirement that the nucleic acid encode the same protein or a common epitope with the influenza nucleoprotein. Since the experiment was never performed, its statement that an effective immune response would be that where the proliferative response of splenocytes would be increased at least two fold, is not even an assertion that any such improvement would be expected, much less there being any data to show this.

Even if this prophetic experiment were performed it lacks controls needed to establish a successful result. The effect of complex is to be compared only to the effect of peptide alone. There is no suggestion to compare the complex with nucleic acid delivered alone, nor is there any suggestion to use the same peptide and nucleic acid administered in other than a complex form. There is no comparison between nucleic acid complexed with peptide of a different type from that encoded and vice versa. For instance it may very well be the case that the nucleoprotein component directs the DNA to the nucleus, and its effects on the immune reaction are non-existent or irrelevant.

Even if the proposed experiments of Craig were to be carried out, therefore, they would not predict that the combination of nucleic acids encoding a common epitope with the peptide had any surprising benefit. For instance it is not possible to dismiss the possibility that the nucleic acid is merely acting as an adjuvant for the peptide antigen.

This is in stark contrast to the working example in the present application described in Example 1. As shown in Table 2 on page 29, all the appropriate controls were included in this experiment. It was shown conclusively (see page 31) that administering an expression system for an epitope shared with that of a protein administered in the same liposome give better results than any of the controls. This is true of cases wherein separate liposomes were used, where the nucleic acid and protein did not contain the same epitope or where liposomes were not used as delivery vehicles.

In summary, Craig describes no tests which involve nucleic acid encoding a peptide sharing the same epitope with the complexing protein. Craig shows no *in vivo* results. Craig has no suggestion to use a nucleic acid encoding a peptide from an infectious organism. Craig does not give any hypothetical or real examples of liposomes. Craig does not suggest experiments which would enable a comparison to be made of the combination of nucleic acid encoding peptide sharing the same epitope with protein, to show any surprising benefit either *in vitro* or *in vivo*. Thus even if the directions of Craig to carry out the hypothetical examples were to be followed, the results would not lead to any expectation of success in the particular combination of features in the claims of the present application.

Taken for what Craig teaches as an entire document, Craig teaches away from the invention because it merely requires nucleic acid and protein (not sharing any epitope) be delivered specifically to an antigen presenting cell. Applicants, on the other hand, have demonstrated that in order to obtain an enhanced immune response, both the nucleic acid and the assistor protein must share an epitope and be provided associated with liposomes together. Enhanced humoral response is not obtained when the nucleic acid and protein are not associated with each other or with

liposomes or do not share the same epitope. Craig would not have predicted this result; Craig teaches away from it by virtue of teaching that such association and such a sharing of epitope, while possible, does not matter.

The Combination with Gregoriadis

Craig was cited in combination with Gregoriadis; however, Gregoriadis was cited only to show that liposomes of various sizes can be prepared and used to encapsulate nucleic acids and/or proteins. Applicants do not rely on the size limitations to confer patentability, and thus Gregoriadis is irrelevant to the invention as claimed.

Claim 27

Further, as illustrated in Example 6 beginning on page 53, the results of this working example show that successful enhancement of the humoral response may be obtained using liposomes which lack phospholipids. This is a requirement of claim 27 and is clearly not suggested by Craig, even in combination with Gregoriadis. Applicants appreciate that this claim is not rejected over the art.

Conclusion

The claims have been amended to obviate the rejections under 35 U.S.C. § 112.

With regard to the art rejection, Craig is not asserted to anticipate claim 13 and in effect teaches away as Craig fails to suggest the necessity for simultaneous interaction with the same cell, of both the nucleic acid and the protein or the necessity for a shared epitope encoded by the nucleic acid and contained in the protein, and, in contrast to the present invention, teaches the necessity to

target APC. In view of the focus of Craig on a cellular as opposed to humoral response, the lack of working example support even for this postulated T-cell response, the failure of Craig to recognize the necessity for co-encapsulation in the same vehicle or for epitope sharing and the assertion by Craig that only professional APC must be targeted, applicants believe Craig fails to suggest the invention as claimed. Even the prophetic *in vivo* example lacks adequate controls to predict the surprisingly improved results demonstrated in actual experiments by Applicants. The present specification provides ample data in support of claim 13, which now excludes targeting to professional APC, requires co-encapsulation, requires a shared epitope and exhibits a humoral immune response, and does not require expression only in APC. The teaching of Gregoriadis is not relevant as applicants do not rely on the features taught by Gregoriadis for patentability.

In view of this, applicants believe that claims 3, 16, 25-26 and 28-30 are in a position for allowance.

Further in view of the amendments that dispose of the rejections under 35 U.S.C. § 112 and the lack of a rejection of claim 27 over the art, it is believed clear that claim 27 is in a position for allowance. Passage of all pending claims to issue is respectfully requested.

Should minor issues remain that might be resolved by phone, a call to the undersigned would be appreciated.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 429022000800.

Respectfully submitted,

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